FACTORS DETERMINING THE DELAY OF TB DIAGNOSIS AND ITS EFFECT ON THE DISEASE TRANSMISSION IN YEMEN

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BY

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Dedication

I dedicate this research work to my beloved parents, my wife and my daughters Sarah, Hagar and Dua'a.

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LIST OF ABBREVIATIONS

AIDS = Acquired Immuno- deficiency Syndrome

AFB = Acid Fast Bacilli

ALA = American Lung Association

BC = Before Christ

CDC = Central Of Disease Control

CI = Confidence Intervals

CT = Computerised Tomography

DNA = Deoxyribo Nucleic Acid

DOTS = Directly Observed Treatment, Short course

ELISA = Enzyme-liked Immunosorbent Assay

EPTB = Extra-Pulmonary Tuberculosis

ETH = Ethambutol

FNA= Fine Needle Aspiration

HBCs = High Burden Countries

HIV = Human Immunodeficiency Virus

INH = Isoniazide

IRIN = Integrated Regional Information Networks

IUATLD = International Union Against Tuberculosis and Lung Disease

IS = Insertion Sequence

MDR = Multi-Drug Resistant

MOH = Ministry Of Health

NTP = National Tuberculosis Program

NTCP = National Tuberculosis Control Program

OR = Odds Ratio

PAS = Para-Amino Salicylic Acid

PCR = Polymerase Chain Reaction

RFLP = Restriction Fragment Length Polymorphism

RIT = Research Institute of Tuberculosis

RNTCP = Revised National Tuberculosis Program

PPD = Purified Protein Derivative

PTB = Pulmonary Tuberculosis

R = Rifampicin

SM = Streptomycin

TB = Tuberculosis

TU = Tuberculin Unit

USD = United States Dollar

WHO = World Health Organization

Z = Pyrazinamide

ZN = Ziehl Neelsen

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FAKTOR-FAKTOR YANG MENENTUKAN KELEWATAN DIAGNOSIS PENYAKIT TB DAN KESANNYA KE ATAS TRANSMISI PENYAKIT DI YEMEN

ABSTRAK

Tuberkulosis (TB) masih kekal sebagai punca utama kematian dan tahap kesihatan yang lemah dengan anggaran 8.8 juta kes baru dilaporkan di seluruh dunia. Kadar kes TB seluruh dunia semakin meningkat pada kira-kira 1.1% setiap tahun dan bilangan kes pada 2.4% setiap tahun. Kelambatan dalam mendapatkan rawatan TB telah meningkatkan lagi transmisi tuberkulosis pulmonari (paru-paru), dan beban penderitaan menanggung penyakit ini masih dirasakan di negara-negara sedang membangun. Kelewatan dalam mengesan TB juga boleh membawa kepada kadar kematian yang semakin meningkat. Walaubagaimanapun, pengesanan awal dan rawatan serta-merta adalah penting dalam pencegahan dan kawalan TB yang berkesan. Tujuan kajian ini ialah untuk menilai kelewatan dalam pengesanan TB dan kesannya ke atas jangkitan TB, sebagai tambahan kepada kesannya ke atas kos pra-diagnosis. Kajian telah dijalankan dalam 3 fasa di sebuah pusat rujukan tuberkulosis di bandar Sana'a, Yemen. Fasa Pertama menjurus kepada pengenalpastian umum tentang masalah kelewatan dan faktor-faktor yang berkaitan. Fasa Kedua bertujuan membuat penilaian ke atas kesan kelewatan ke atas jangkitan TB di kalangan keluarga atau rakan-rakan rapat. Dalam Fasa Ketiga, hubungan di antara kos pra-diagnosis dan kelewatan diagnosis ke atas pesakit-pesakit TB telah dinilai. Kira-kira 505 pesakit TB dengan lumuran positif yang baru telah dilatih untuk kajian ini. Kesemua 505 orang pesakit telah diminta menjawab soal kajiselidik dan temuramah. Seterusnya, 266 rakan dan keluarga terdekat telah menjalani Ujian Penyaringan Tuberkulin (TST). Alat TST ini telah digunakan dalam fasa kedua kajian ini.

Keputusan-keputusan yang diperolehi dalam Fasa Pertama menunjukkan bahawa, kelewatan median adalah berjumlah 60 hari, 30 hari untuk kelewatan pesakit dan 10 hari untuk kelewatan penjagaan kesihatan. Faktor risiko bebas untuk jumlah kelewatan yang panjang ialah jantina lelaki, sudah berusia (> 60 tahun), mempunyai status pekerjaan, mula mendapatkan rawatan awal di farmasi, rawatan yang tidak betul, iaitu dengan antibiotik, bilangan lawatan ke unit penjagaan kesihatan dan *cauterization* sebagai rawatan tradisional. Untuk kelewatan pesakit yang panjang (> 30 hari) faktor ialah usia tua (> 60 tahun), rawatan yang tidak betul dengan antibiotik, Farmasi sebagai tempat pertama dilawati oleh pesakit-pesakit TB, status ekonomi, kesukaran mendapatkan kenderaan pengangkutan dan kelapangan suami. Untuk kelewatan penjagaan kesihatan yang lama (> 10 hari) faktor-faktornya ialah rawatan yang tidak betul dengan antibiotik, bilangan lawatan ke unit penjagaan kesihatan dan aras rendah TB *bacilli* dalam lumuran pesakit TB. Dalam Fasa Kedua, kelainan dalam pola transmisi TB di kalangan orang terdekat tidak dapat diperolehi. Dalam Fasa Ketiga, perbezaan kos pra-diagnosis dapat direkodkan. Perubatan secara langsung (kos perubatan, ujian makmal dan ujian Sinar-X Dada) dan faktor bukan-perubatan secara langsung (kos makanan, pengangkutan dan tempat tinggal) yag ditanggung oleh pesakit-pesakit TB adalah berbeza ($P < 0.001$). Telah dapat disimpulkan bahawa faktor-faktor berkaitan pesakit dan sistem penjagaan kesihatan telah menyumbang secara signifikan kepada kelewatan dalam diagnosis atau pengesanan TB. Lelaki, usia yang tua (> 60 tahun), status pekerjaan, mendapatkan rawatan di farmasi, rawatan yang tidak sempurna dengan antibiotik, bilangan lawatan ke unit penjagaan kesihatan dan *cauterization* adalah faktor-faktor risiko untuk kedua-dua pesakit dan sistem penjagaan kesihatan, sementara status ekonomi dan kesukaran mendapatkan pengangkutan hanyalah faktor-faktor risiko kelewatan

pesakit, dan aras tinggi TB *bacilli* adalah faktor risiko kepada kelewatan sistem penjagaan kesihatan. Perbezaan dari sudut transmisi TB di kalangan orang terdekat kepada pesakit yang lewat atau tidak terlewat dikesan tidak dikaji. Pesakit-pesakit yang lambat dikesan terpaksa membayar kos tambahan sebelum pengesanan sebenar TB dilakukan. Meningkatkan pengajaran dan kesedaran tentang TB di kalangan rakyat dan sistem penjagaan kesihatan telah dicadangkan dalam kajian ini.

FACTORS DETERMINING THE DELAY OF TB DIAGNOSIS AND ITS EFFECT ON THE DISEASE TRANSMISSION IN YEMEN

ABSTRACT

Tuberculosis (TB) still remains a leading cause of mortality and morbidity with an estimation of 8.8 million new cases in the world. The global incidence rate of TB was growing at approximately 1.1% per year and the number of cases at 2.4% per year. Delay in seeking care of TB increase transmission of pulmonary tuberculosis as well as the burden of TB, which remains high in developing countries. This delay in the diagnosis of TB also could result in increased patient morbidity and mortality. However, early diagnosis and immediate initiation of treatment are essential for an effective TB prevention and control. The aim of this study was to evaluate the delay in TB diagnosis and its effect on TB transmission in addition to its effect on pre-diagnosis cost. The study was carried out in 3 phases in a referral centre of tuberculosis at Sana'a city, Yemen. Phase One was concerned of a general identification of delay problem and its associated factors. Phase Two was aimed to assess the effect of delay on TB transmission among close contacts. In Phase Three, the relation between pre-diagnosis cost and delay diagnosis of TB patients was assessed. About 505 new smears positive TB patients were recruited to this study. All of the 505 were subjected to interview questionnaire. Moreover, 266 close contacts were subjected to Tuberculin Screening Test (TST). This TST tool was used in phase two of this study. The results obtained in Phase One revealed that, median delay was 60 days for total, 30 days for patient and 10 days for health care delay. Independent risk factors for long total delay were male gender, old age (> 60 years), employment status, seeking care at Pharmacy as first place visited by TB patients,

improper treatment with antibiotics, number of visit to health care unit and cauterization as traditional treatment. For long patient delay (> 30 days) these were old age (> 60 years), improper treatment with antibiotics, Pharmacy as first place visited by TB patients, economic status, transportation difficulties and availability of husband. For long health care delay (> 10 days) these were improper treatment with antibiotics, number of visit to health care unit and low level of TB bacilli in smears of TB patient. In Phase Two, the difference in TB transmission pattern among those close contacts could not obtain. In Phase Three, pre-diagnosis cost differences were obtained. Direct medical (medication cost, laboratory investigation and Chest X-Ray test,) and direct non-medical (food, transportation and accommodation) costs that incurred by TB patients were different ($P < 0.001$). It was concluded that patient and health care system related factors contribute significantly to delays in the diagnosis of TB patients. males gender, old age (> 60 years), employment status, seeking care at Pharmacy, improper treatment with antibiotics, number of visit to health care unit and cauterization were risk factors of both patient and health care system while economic status and Transportation difficulties were only risk factors of patient delay and high level of TB Bacilli was risk factor of health care system delay. The difference in terms of TB transmission among close contacts of delay and non delay patients was not observed. Delayed diagnosis patients were incurred extra costs prior to actual TB diagnosis. Promoting knowledge and awareness of TB among both population and health care system in public and private sector was recommended.

CHAPTER ONE

INTRODUCTION

1.1 Tuberculosis as a worldwide problem

Tuberculosis (TB) is one of the most profound health problems worldwide. With the recent resurgence of TB and multidrug resistant cases, TB control, which depends on rapid identification of TB infection as well as case finding and effective treatment, is threatened. Therefore, TB remains as one of the complicated problems faced by the world today. It is a chronic communicable continues resistant disease caused by microorganism known as *Mycobacterium tuberculosis* (Yilmaze *et al.*, 2004; Schwartzman, 2002).

Tuberculosis is as old as mankind and the history of this disorder is intertwined inevitably with the history of civilization. It is also thought to be the oldest of human diseases. Many people think of TB as a disease of the past. But, TB is still a leading killer of young adults (productive age group) worldwide. Some 2 billion people (one-third of the world's population) are infected with the TB bacterium, *Mycobacterium tuberculosis* (CDC, 2008; Jenkins, 1995). It is a disease which is spread through the air and usually infects the lungs although other organs are sometimes involved. This includes the lemphnodes, bones, kidnys, abdomen, genito-urinary tract, skin, joints and brain (Anderson and Muirs, 1985; Lucaya and Strife, 2002). Pulmonary tuberculosis usually occurs in the apex of the lung. This develops cavities containing large numbers of tubercle bacilli, which can be detected in sputum specimen (Janic, 2003).

Most people who are infected with *Mycobacterium tuberculosis* harbour the bacterium without symptoms while others develop active TB disease. Each year, 8 million people worldwide develop active TB with 3 million deaths (Paul *et al.*, 2000).

TB is a global emergency. Data obtained from the World Bank reveals that TB kills more adults than any other single infectious agent. Additionally, TB prevention and control interventions are among the most cost-effective public health measures (Ramachandaran and Paramasivan, 2003). Despite the magnitude of the TB control strategies, the disease still remains a major public health challenge especially in the developing world. Socio-economic problems are considered as complicated challenges of patient's easy access to TB diagnosis and treatment. Conducting of well national prevalence survey could assist in understanding such challenges as well as in combating TB threatens (Omoleke, 2012).

1.2 Epidemiology of TB

Tuberculosis continues to be a very major problem throughout the world. A more reasonable estimate of the total number of new cases each year is 5.5 million, of which 74% occur in Asia and another 12% in Africa (International Union Against Tuberculosis and Lung Diseases, IUATLD, 2000). About 95% of deaths due to TB are in the developing countries (Sajjad *et al*., 2003). While the incidence of tuberculosis has declined considerably in industrialized countries, the disease still poses a serious and even increasing problem in many low-income countries, affecting the health and social welfare of millions of people. Fighting tuberculosis is a challenge to all who are concerned about health and development (Loddenkemper *et al*., 2002).

The trend in case notification (Thomas *et al*., 2003) suggests that the global incidence of tuberculosis is growing, although slowly, i.e. 0.4 % cases/ year (Health Protection Agency, 2003). Much more rapid growth of the disease incidence is found in sub-Saharan Africa which is linked to the spread of HIV. The rapid growth of incidence is also found in countries of the former Soviet Union due to the deterioration of public health and public health services (WHO. 2005a). Meanwhile, the incidence of tuberculosis continues to decline in western and central Europe and in other industrialized countries, and more slowly in Latin America and the Middle East (WHO. 2005a).

According to WHO report (2011), the TB incidence rate was falling or stable in all six WHO regions (the Americas, Eastern Mediterranean, Europe, South-East Asia, Western Pacific and Africa, Table 1.1). The falling incidence's rate was by 1.3% per year since 2002.

Table 1.1: Estimated TB incidence, prevalence and mortality of 2010.

WHO region	Incidence[1]		Prevalence [2]	Mortality	
	No. in thousands	% of global total	No. in thousands	No. in thousands	Rate per 100 000 pop[3]
Africa	2300	26%	2800	250	30
The Americas	270	3%	330	20	2.2
Eastern Mediterranean	650	7%	1000	95	16
Europe	420	5%	560	61	6.8
South-East Asia	3500	39%	5000	500	27
Western Pacific	1700	20%	2500	130	7.5
Global total	8800	100%	12000	1100	15

[1] Incidence is the number of new cases arising during a defined period.
[2] Prevalence is the number of cases (new and previously occurring) that exists at a given point in time.
[3] Population

The highest number of death was in the South East Asia Region while the highest mortality per capita was in the African Region. The decline in the incidence rate per capita was offset by population growth. Thus, the number of new cases arising each year is still increasing globally in Africa, the eastern Mediterranean and South East Asia (WHO, 2011).

1.3 Microbiology of TB

Tuberculosis affects everyone from infants to elderly age. Tuberculosis can spread through droplet infection or contaminated foods, such as milk from herds infected with *Mycobacterium bovis* (National Collaborating Centre for Chronic Conditions, 2006). Bovine tuberculosis has been almost completely eliminated following the pasteurization of milk and the control of infected cattle. Unfortunately, there is a reservoir in wild species and it is known to have infected buffaloes and re-infection of domestic cattle is thus always possible (Spalmer *et al.*, 2002).

Tuberculosis originally spread from animal to humans, just as AIDS did in the twentieth century. It probably leaped from cows to humans about 8,000 or 10,000 years ago, when people first settled down in communities to tend to their cattle and plant their crops. From their cows, some farmers and their families probably contracted an airborne infection from *Mycobacterium bovis*. Sick cows exhaled the bacteria, and human beings breathed them in. They may also have got the tuberculosis bacteria from the cows' milk. The bacteria learned to live in the human body, which is not that different from a cow, preferring to settle in the lungs, although they could attack many other organs (Reichman, 2002).

The infectious agent of TB (*M. tuberculosis*) is a thin, slightly curved bacillus that is an obligate aerobe. In comparison to other bacteria, *M tuberculosis* has a cell wall with very high lipid content (Kathleen and Arther, 2002; Leung, 1999) that resists staining by the usual Gram method. The unique character of mycobacteria is its acid fastness property (Bruce *et al.*, 1999). Once stained by an aniline dye such as carbol fuchsin, it resists decolourisation with acid and alcohol and thus is termed as acid and alcohol-fast bacilli (AAFB). This was then shortened to 'acid fast bacilli' or AFB (Jenkins, 1995; Palomino *et al*., 2007).

Mycobacterium tuberculosis also considered as a non-spore forming, non-motile and non-encapsulated bacillus.

Acid fastness is related to the uniquely thick cell wall, which is composed of an interlacing layer of lipids, peptidoglycans and arabinomannas. The aniline dye forms a complex with this layer and is held fast despite the action of the acid-alcohol. This allows the detection of AFB in the specimen, with a simple staining technique called the Ziel-Neelson (ZN) stain, which has been in use for over 60 years (Monica, 2000).

1.4 Pathogeneses of TB

Inhaling cough droplets or dust particles containing tubercle bacilli can causes infection with *Mycobacterium tuberculosis*. These bacilli can be lodged in the lung to form a small inflammatory lesion (WHO, 2004). The bacilli also infect adjacent lymph nodes (Monica, 2000). Activated macrophages form a granuloma around the site of primary infection, which usually are response (macrophage) to the microbial antigen. The immune system cells (mononuclear cells) are recruited to the site of the infection. Then the T immune cells produce cytokines, which act on the macrophage containing the bacilli at the centre of the granuloma, and control the infection. Spread of infection is prevented by confining the bacilli inside the granuloma (Gerd-Rudiger and Antonio, 2003).

The T cells with the activated macrophage work together to isolate and inhibit the replication of the bacilli inside the granuloma. Then, the immune cells produce toxic substance to inhibit and destroy the bacilli inside granuloma (Monica, 2000; Palomino *et al*., 2007). In most patients the primary lesion is self-healing although not all the bacilli may be destroyed and some bacilli remain dormant in

lymph nodes and may reactivate causing post-primary disease. There may be fibrous scarring and sometimes calcification of the healed area. Unfortunately, some bacilli may still survive inside granuloma and may reactivate to cause re-infection years later (Yancey, 2001).

Tuberculosis has two general status: latent infection and active disease (WHO, 2004). Only those who develop active tuberculosis can transmit the disease. The difference between latent tuberculosis and active pulmonary tuberculosis is that the presence of symptoms, presence of tubercle bacilli inside sputum sample and the abnormal chest X- ray result are usually found within the active TB case. Latent infection refers to the remainder of the bacilli inside the body without any reactivation (Marilyn, 2001; Schwartzman, 2002). The mycobacterium with active tuberculosis (infectious status) will be transmitted from the infected person to another depending on several factors which include:

- The infectiousness of the person with infectious tuberculosis, which is related to the number of bacteria that he or she expels and possibly the virulence of the bacteria.
- The length of exposure to an infectious person or to air contaminated with tuberculosis bacteria (long time exposure leads to probability of disease risk).
- The environment surrounding an infectious person, for example, the size of a room and how well the ventilation is inside the room.
- The functioning of an exposed person’s immune system (status of immunity of the exposed person).

1.5 High-risk groups

The following groups are the high exposure groups to tuberculosis infection, (CDC, 2001; American Lung Association, 2011):

1 - Close contact of persons known as tuberculosis- infected persons or persons suspected of having TB (e.g. family and friends).

2 - Persons infected with HIV.

3 - Persons who inject drugs or other locally identified high–risk substance users (e.g. crack cocaine users).

4 - Persons who have medical risk factors known to increase the risk for disease if infection occurs (diabetes, asthma, immunosuppressive people).

5 - Residents and employees of high-risk congregate settings (e.g., correctional institutions, nursing homes, mental institutions, other long-term residential facilities, and shelters for the homeless).

6 - Health-care workers, who serve high-risk clients.

7 - Foreign-born persons, including children, recently arrived (within 5 years) from countries that have a high TB incidence or prevalence.

8 - Some medically underserved persons (poor medical service) and low-income populations.

9 - High-risk racial (some races are at more risk of contracting tuberculosis) or ethnic minority populations, as defined locally; and infants, children and adolescents exposed to adults in high-risk categories.

1.6 Tuberculosis transmission

The source of TB infection (droplet nuclei) is carried to the surrounding environment by the air from the infected cases (Jacques, 2003). In this way, the disease is spread from person to person. *Mycobacterium tuberculosis* is contained in small particles (droplets) range from 1 to 5 μm in diameter. The droplet nuclei are produced when persons with pulmonary tuberculosis cough, sneeze or speak (Lawrence, 2000; Rhee, 2001). The droplets may also be produced by aerosol treatments, sputum induction, and aerosolization during a bronchoscope and through manipulation of lesions or processing of tissue or secretions in the hospital or laboratory (Robert *et al*., 2000). Droplet nuclei contain two to three *Mycobacterium tuberculosis* organisms. The organisms are so small that air currents normally present in any indoor space can keep them airborne for long periods of time. The droplet nuclei are small enough to reach the alveoli within the lungs, where the organisms replicate (Reichman and Hershfield, 2000; Golub, 2002). Although patients with tuberculosis can generate larger particles containing numerous bacilli, these particles do not serve as effective vehicles for transmission of infection because they do not remain airborne. These particles also do not reach the alveoli (if inhaled). Organisms deposited on intact mucosa or skin does not invade tissue. When large particles are inhaled, they impact on the wall of the upper airways where they are trapped in the mucous blanket, carried to the oropharynx, and swallowed or expectorated (Respire, 2000).

The transmission of TB can be limited if the disease is diagnosed in a timely manner (Yilmaz *et al.*, 2004). The delay in diagnosis of TB can lead to increase morbidity and mortality beside persistence of the patients as a reservoir of disease

transmission. Delay in the diagnosis may have serious implication at both the individual and the community level (Olumuyiwa and Joseph, 2004).

1.6.1 Tuberculin test and TB transmission

Epidemiologically, Tuberculin Skin Test (TST) is used to investigate the transmission of TB among contact (ALA, 2011).The interpretation of the result would indicate if the contact is infected or not. TST positive result will indicate that the contact is infected with TB. This infected contact with TB may reactivate to become a TB patient any time (CDC, 2008).

In a population with high prevalence of TB, Tuberculin test is more reliable due to the high specificity and high sensitivity level of the test in terms of detecting the disease (American Thoracic Society, 1999).

Contact investigations for patients with TB are initiated to identify secondary infections and cases. This is because; TB patient with delay diagnosis will give a greater chance for TB to transmit to contact (Golub, 2002). The rationale for conducting contact investigation is based on two key principles of TB control: 1) to treat all cases of disease and 2) to reduce the overall rate of the disease (Rhee, 2001).

Recently, molecular epidemiology is involved in the assessment of disease transmission pattern among contacts. In a practical application of the techniques of molecular biology, genetic fingerprinting has been used in epidemiological investigation of tuberculosis transmission (Kulaga, 2004). The (IS6110) restriction fragment length polymorphism (RFLP) typing is used for tracing the spread of *Mycobacterium tuberculosis* strain. This type has been widely used to demonstrate transmission of TB in institutional outbreaks (Diaz *et al.*, 2001).

RFLP-typing is one of many typing techniques that are available in which polymorphisms in the number or location of repetitive DNA elements (insertion sequences) within the bacterial genome can be used in epidemiologic study. An insertion element is a piece of DNA that is able to move independently and to insert in multiple location in plasmids or chromosomal locations. Copies of this insertion sequence (IS) are identical in sequence but their number and location in the genome vary. Person-to-person transmission is inferred on the basis of identical or very similar DNA fingerprints of the bacterial organism (Kulaga, 2004).

1.7 Diagnosis of TB

The diagnosis of TB usually depends on laboratory investigation methods, radiographic chest-X rays and the clinical symptoms of the patients (Palomino, 2007).

1.7.1 Diagnosis of pulmonary TB

The lungs are primarily infected with *Mycobacterium tuberculosis*. Pulmonary TB (PTB) develops in the minority of people whose immune systems do not successfully contain the primary infection. The disease may occur within weeks after the primary infection, or it may lie dormant for years before causing the disease (Darrell, 2002).

PTB is the common form of tuberculosis in adults. This form (PTB) is the only form of tuberculosis which can be infectious and thus has a great epidemiological significance (WHO, 2004).

1.7.1.(a) Signs and symptoms

The symptoms which suggest pulmonary tuberculosis include:

- Cough persisting for more than three weeks.
- Loss of weight and loss of appetite.
- Fever.
- Dyspnoea (shortness of breath), night sweats, chest pain and hoarseness of voice, all of which are uncommon.

Patients with the above symptoms should be screened for tuberculosis (WHO, 2001a). Signs can be subtle especially in minimal cases, or may be obvious including those of consolidation, fibrosis or stony dullness caused by pleural effusion.

1.7.1.(b) Investigations

Diagnosis of suspected TB patients is usually performed via the TB laboratory investigations, performing chest-x ray in addition to sign and symptoms of TB that are confirmed by TB doctors and specialists.

1.7.1.(b).(i) Laboratory investigation

Laboratory tests should be used to improve the outcome for individual patients or to provide public health information. However, if the quality of laboratory tests is poor, resources will be wasted on repeat tests or inappropriate management and the laboratory service will be inefficient (Mundy *et al.,* 2003).

Sputum smear microscopy - Acid fast staining

For the diagnosis of tuberculosis, the sputum smear microscopy has traditionally been the first diagnostic test used to screen for active PTB disease. The

smear is usually stained using the acid fast staining method (Michael *et al.*, 2001). The acid-fast smear can be performed quickly and can provide information to the clinician in less than 24 hours. However, it has several limitations. These limitations include the difference of examiners competency in terms of conducting of the AFB test, the way of collecting the sputum samples by patients and preparing the samples by the technicians (Josephine *et al*., 1998).

For sputum smear microscopy, three specimens of sputum are required preferably including one early morning specimen (Peter, 2000).

Culture test

Culture of the organism can be performed on a conventional egg-based media such as Lowenstein-Jensen medium or Middle Brook 7H10 medium. Growth on these conventional media takes 4-8 weeks to obtain results (Ganguly *et al.*, 2002a). However, radiometric culture method such as the BACTEC method can provide results within 2 weeks. This method can be used when there is a need for early diagnosis and in smear-negative cases. This method depends on C^{14} labeled palmitic acid which can be utilized by the Mycobacteria, and the radioactive carbon dioxide released will be an indicator for the presence of the bacilli (Monica, 2000). Sensitivity tests using radiometric method which yield equally rapid results can be performed in situations where drug resistance is suspected and urgent results are required (MOH, Malaysia, 2002).

Polymerase chain reaction (PCR) technique

This method depends on the nucleic acid amplification technique. The PCR allows sequences of DNA present in a few copies of mycobacteria to be amplified *in*

vitro, which can be visualized and identified (Padmavathy *et al*., 2005; CDC, 2008). Although PCR technique can give rapid result, it is expensive. False positive reaction may result from cross-contamination (MOH, Malaysia, 2000). The false negative results may also come from the presence of inhibitor or lack of target gene sequences in the causative strain. It must be remembered that PCR can also give a positive result in patients who are already on anti-tuberculosis treatment but who are excreting small numbers of non-viable bacilli. Thus, this test cannot be used for follow-up on patients whose are under treatment (Joseph, 2000).

Serological test

Most of the serological tests for diagnosis of TB have high negative predictive value or low sensitivity to identify the disease and thus are useful as screening tests. Numerous serological tests that use various antigens, such as secreted and heat shock protein lipopolysaccharides and peptides have been developed (Sudha *et al*., 2000). Serological tests for tuberculosis should be studied in various operational situations before they are widely applied. The limitation of these tests is the low sensitivity especially with smear negative patients and also with HIV positive cases. Additionally, another difficulties relating to the distinguishing between *M. tuberculosis* and non-tuberculosis mycobacteria were due to the similarity of the content of these TB bacilli organisms (Gunguly *et al.*, 2002).

1.7.1.(b).(ii) Mantoux or tuberculin test

This test (Tuberculin) is reliable for the diagnosis of tuberculosis in the asymptomatic individual (Michael, 2001). It is an extract of the tubercle bacilli, which aids in identifying TB infection (Sudha, 1999; CDC, 2008). Tuberculin is

administered intra-dermally with 5-tuberculin units. The positive results appear as induration around the injection (more than 10 mm in diameter). The result can be read within the time frame of 48-72 hours (William, 2001).

Tuberculin test has a role in the diagnosis of tuberculosis especially in pediatric cases and cases of extra pulmonary tuberculosis. The Mantoux test is carried out in government hospitals in Malaysia using two tuberculin units (T.U) in 0.1 ml prepared solution (buffer solution). Diameter of indurations of less than 10 mm is graded as negative. When the reading result is 10 mm or more (in a child or adult) it should be considered as a positive result. The diameter of 15 millimeter or more in children is considered as significant for TB and may indicate recent infection. A positive Mantoux test merely indicates tuberculosis infection and not necessarily active disease. (MOH, Malaysia, 2002; CDC, 2008).

The tuberculin test can represent a true infection in the population who are only infected with *Mycobacterium tuberculosis* and also those who are not vaccinated with BCG. When people infected with other mycobacteria species are subjected to tuberculin test, the false positive result will appear as a cross-reaction among them. This cross-reaction (false positive result) occurs due to the reaction between the antibodies of TB with similar antigens of mycobacteria rather than *M. tuberculosis* antigen.

1.7.1.(b).(iii) Chest X- ray

The classic description of the chest radiograph of adults with TB is that of "post primary" TB, with nodular or alveolar infiltrates in the upper lung zones (the apical and posterior segments of the upper lobes and the superior segment of the lower lobes) and evidence of cavitation. This is still a common mode of presentation

and, if present, the physician should be prompted to consider the diagnosis. Surprisingly, several studies have shown that up to one-third of patients with this classic chest radiographic findings are not initially suspected of having TB due to many other lung diseases that may mimic the existence of pulmonary TB (Richard and Robert, 1999).

The lesion of TB is revealed in the apical and posterior segments of the upper lobes. Lesions are often soft in active pulmonary tuberculosis and there is usually little or no fibrosis or calcification. These findings would suggest healed tuberculosis. Cavities suggest the diagnosis of active disease unless the patient has been previously treated. Apart from the typical sites at the apices, other sites such as the apical segment of the lower lobes may also be involved (MOH, Malaysia, 2002).

Asnake and Feleke (2000) reported that the radiological diagnosis of TB is unreliable, since many other lung diseases may mimic the existence of pulmonary TB. In addition, the cost of radiographic examination is high and very often not affordable for all cases of TB in low-income countries (WHO, 2005b).

1.7.2 Diagnosis of extra Pulmonary TB (EPTB)

Tuberculosis of organs other than the lungs is called extra pulmonary tuberculosis. EPTB includes the pleura (TB pleurisy), peripheral lymph nodes, abdomen, genito-urinary tract, skin, joints, bones, and the brain (National Collaborating Centre for Chronic Conditions, 2006).

Diagnosis of EPTB is often difficult. A negative smear of acid-fast bacilli and failure to culture the bacilli contributed to the difficulty of EPTB diagnosis (Marjoriep, 2005). Thus, the recognition and understanding of the radiographic findings in the EPTB case can reduce the difficulty of EPTB diagnosis (Engin *et al*.,

2002). In the post AIDS era (Marco *et al*, 1998) in USA, 1008 patients with extra pulmonary tuberculosis were older than 65 years old. Two thirds of the 4 million infected people with both HIV and TB were diagnosed as having extra pulmonary tuberculosis. The diagnosis of EPTB tuberculosis is often difficult compared with pulmonary TB due to the low sensitivity of the usual diagnostic methods for the disease (AFB smear, Mantoux, culture and chest radiography tests). The sensitivity of the previous mentioned methods ranges from 25% - 39% (Marco *et al.*, 1998).

1.7.3 Lymphadenitis TB

Tuberculous lymphadenitis is the most common type of extra pulmonary tuberculosis (CDC, 2008). It can be diagnosed by fine needle aspiration (FNA) of glands (lymph nodes glands). This type of specimen is accessible especially in the neck. The specimen is submitted for AFB test and culture and cytological examination. The biopsy specimens may be needed in case of negative FNA test results (Sharma and Mohan, 2004).

1.7.4 Pleural effusion TB

In certain parts of the world, tuberculosis remains as the most frequent cause of pleural effusion in the absence of an obvious pulmonary lesion. Diagnosis of tuberculosis in pleural effusion can be made by pleural effusion culture. The biopsy can also be used as a specimen to diagnose TB. When the microscopic examination of the biopsy specimen is combined with the culture, the diagnosis can be made in 90% of patients, especially with the use of multiple biopsies (Wilfredo *et al.*, 2000; WHO, 2004).

1.7.5 Skeletal TB

Skeletal tuberculosis typically involves the vertebrae as well as the weight-bearing bones and joints. The early diagnosis for the disease is critical to the preservation of the cartilage and joint space. The diagnosis is performed by a radiographic test. The lesion can be evaluated well by CT scanning or magnetic resonance imaging (MRI). Unfortunately, these methods are not made available for all tuberculosis patients. The tissue biopsy test should also be required especially with tuberculosis in the intestine and peritoneum (Sharma and Mohan, 2004; Palomino *et al*., 2007).

1.7.6 Genito-urinary TB

The genito-urinary tract is the second most common site for tuberculous infection after the lungs as reported by Khan and Chandramohan (2011). According to Chattopadhyaya *et al.*, 1997, it has been reported to account for 20% to 70 % of all cases of extrapulmonary tuberculosis. The infection almost always affects the kidneys during the primary exposure to infection but is not presented clinically (Chattopadhyaya *et al.*, 1997). Here the diagnosis of tuberculosis can be made with collection of three to six consecutive morning clean urine specimens for culture; Radiological investigation can also be made in this case (Wilfredo *et al.*, 2000).

1.7.7 Miliary TB

Miliary TB may occur in an individual organ (very rare, < 5%). It also occurs in several organs of the body or throughout the whole body (> 90%), including the brain. The infection is characterized by the presence of a large amount of TB bacilli. Despite the presence of the large amount of bacilli the disease may be misdiagnosed.

Up to 25% of patients with miliary TB may have meningeal infection. In addition, miliary TB may mimic many diseases. In some case series, up to 50% of cases are undiagnosed antemortem (Klaus and De Luise, 2011). Therefore, the high index of clinical suspicion is important to obtain an early diagnosis and ensure improved clinical outcomes. The diagnosis of this type of tuberculosis can be supported by chest X-rays. The liver biopsy, cerebrospinal fluid test required especially in tuberculous meningitis and CT scans in brain tuberculosis may also help to assess suspected TB lesions (National Collaborating Centre for Chronic Conditions, 2006).

1.8 Drug resistance *Mycobacterium*

When streptomycin was discovered in 1943 and introduced as the first antibiotic for the treatment of TB, the majority of those treated improved dramatically. In most cases, this improvement was followed by a relapse due to streptomycin-resistant strains, and the importance of drug resistance in the treatment of TB became apparent. It is now known that the use of streptomycin monotherapy has led to the emergence of resistant strains. Since drug resistance develops rapidly, inadequate treatment or non-adherence to treatment commonly leads to the development of drug-resistant disease with subsequent transmission of drug-resistant strains within the community (CDC, 2008; Loddenkemper, 2002).

Primary resistance is the resistance pattern seen in new patients who have not been exposed to anti-TB drugs previously. Secondary resistance is the resistance pattern in patients with previous history of anti-TB treatment and ineffective chemotherapy (Heidarnejad and Nagili, 2001).

1.9 Treatment of TB

Early diagnosis and prompt treatment of TB is important in order to minimize complications and the consequences of the disease. To cure the individual patient of tuberculosis, and to minimize the transmission of *Mycobacterium tuberculosis* to other persons are the main two goals of TB treatment. Thus, successful treatment of TB has benefits for both the individual patient and the community in which the patient resides. For this reason, the prescribing physician, be he/she in the public or private sector, is carrying out a public health function with a responsibility of not only for prescribing an appropriate drug regimen but also for successful completion of therapy. However, given a clear understanding of roles and responsibilities, oversight of treatment may be shared between a public health programme and a private physician (American Thoraces Society, 2003).

Before the discovery of streptomycin, fresh air, plenty of food, sunshine and bed rest were the available treatment for TB. Effective chemotherapy was started when streptomycin(SM) was discovered in the 1940s. The resistance for this treatment was developed, and then it was found that combined chemotherapy with streptomycin plus paraaminosalicylic acid (PAS) prevented the emergence of strains resistant to streptomycin (Cynamon, 2000). The response to streptomycin plus PAS treatment was better than the response of single drug TB treatment. The introduction of isoniazide (INH) alone or in combination with SM or PAS in the treatment of pulmonary tuberculosis further confirmed the inadequacy of mono therapy due to the onset of drug resistance. The standard regimen of chemotherapy contained SM plus PAS and had to be given for a minimum of 18 months to 2 years. PAS could be substituted by ethambutol (ETH) according to the TB patient acceptability and availability (Chan, 1995; Palomino *et al*., 2007).

Rifampicin, one of the important drugs in the treatment of TB was able to kill the very slowly dividing bacteria, the so-called "persisters" in a way that the other drugs could not. It was found that by combining this drug with at least two others initially, the length of treatment could be reduced to just six months. As aresult, the new standard of treatment of tuberculosis became isoniazide (INH), rifampicin (R), and pyrazinamide (Z) for two months followed by isoniazide and rifampicin for four months (Peter, 1999).

Shortening the course of anti-tuberculosis treatment to ensure that a person completes treatment has been a major practical tuberculosis control research issue for decades. Highly efficacious regimens now exist that can be completed in just 6 months with as few as 62 doses of anti-tuberculosis drugs (James, 2000). The shortest course regimen required initiation of treatment with four drugs, including isoniazide, rifampicin, and pyrazinamide with either ethambutol or streptomycin as the fourth drug (CDC, 2011). The same regimen that makes short-course treatment a possibility also covers the potential for single- or even two-drug resistance. Thus, the best tuberculosis control strategy for the 1990s dictates initiation of four anti-tuberculosis drugs in nearly all cases (James, 2000).

1.9.1 Treatment regimens

The current recommended TB treatment regimens of the National Tuberculosis Programme (NTP) in each country depends on the particular country’s budget, health coverage by Public Health Centre (PHC) services and qualifications of health staff at peripheral levels (CDC, 2011). For each patient, the regimen recommended depends on the patient treatment category (WHO, 2004). The

treatment programme of TB based on WHO treatment categories is divided into three categories as follows (MOH, Malaysia, 2002):

- New tuberculosis cases.
- Relapse, treatment failure, and treatment after interruption.
- Chronic cases.

Treatment regimen in each category is divided into two phases, namely

A) Intensive phase.

B) Continuation phase.

In the intensive phase, three or four drugs are given daily while two or three drugs are usually given in the continuation phase. In the case of multi-drug resistance patients, trained physicians should do the treatment due to the different treatment time and regimen (long course of anti TB drugs) compared with patients who are infected for the first time. In such cases, it is advisable to manage the patient as an in-patient until sputum conversion (changing of sputum results from positive to negative) is achieved (CDC, 2008; WHO, 2005c). In the management of multi-drug resistant TB, the initial regimen should consist of at least three drugs, preferably four or five, to which the bacilli are likely to be sensitive. When sputum converts to negative, one or more drugs may be withdrawn, preferably a weaker drug which causes side effects. The treatment with the weaker regimen should be continued for at least 18 months after sputum conversion to prevent relapse (Palomino *et al*., 2007).

1.10 DOTS and TB treatment

Directly Observed Treatment, Short course (DOTS) is an international recommended approach to control TB. It is an inexpensive strategy to prevent

millions of TB cases and deaths over the coming decade. The DOT programme is involved in providing the anti-tuberculosis drugs directly to the patient and watching as he/she swallows the medications. It is the preferred core management strategy for all patients with tuberculosis (American Thoracic Society, 2003). Directly Observed Treatment Short Course (DOTS) is a comprehensive strategy endorsed by the World Health Organization (WHO) and International Union Against Tuberculosis and Lung Diseases (IUATLD) to detect and cure TB patients. This strategy of DOTS combines five components (WHO, 1999):

1. Government commitment.
2. Case detection by sputum smears microscopy.
3. Standardized treatment regimen.
4. Regular drug supply.
5. Standardized recording and reporting system.

According to WHO report (2005b), the total number of countries implementing DOTS were increased by two fold during 2003, bringing the total to 182 out of 211 countries around the world. There were 22 countries named as high burden TB countries and they represent 80% of the global burden of tuberculosis. All the 22 High Burden Countries (HBCs) that included in the WHO report have had DOTS programmes since 2000; many of these programmes have been established for much longer. DOTS as programme to control TB has coverage in all countries around the world and its implementation, has been steadily increasing since 1995 (Amdekar, 2005).

1.11 Delay in TB diagnosis

Delays in the diagnosis of tuberculosis (TB) are problems associated with many TB patients around the world; these delays may result in increased patient morbidity and increased of TB transmission (Mathur *et al*., 2001). These delays may reflect patient delays in seeking care, health care provider (HCP) delays in making the diagnosis and starting treatment, or even both patient and HCP delays (Mathur *et al*., 1994; Xu *et al*., 2006). TB patients are characterized according to the time of diagnosis either with short or long diagnostic delay period (Yimer *et al*., 2005), in which the latter period could lead to continuous TB transmission.

Several studies have sought to establish whether the delay in diagnosis of TB is due to a patient delay in seeking care or due to the inability of the provider (health care provider) to diagnose promptly (WHO, 2001b). The literature generally makes a distinction between the two normally, patients delay and health care system delay. The total delay is the main term of TB delay diagnosis and it consisted of the two previous mentioned parts of delay (patient and health care system delay) in addition to treatment delay (WHO, 2006a; Zerbini *et al*., 2008).

According to WHO, 2006a, and other literatures such as Zerbini *et al*., 2008; Nguyen *et al*., 2009, the definitions of these types of delay:

- Patient delay is defined as the time interval between the onset of TB symptoms and first consultation at a health care unit.
- Health care system delay is usually referred to the time between the first visit to the health care unit and actual diagnosis of TB (Nguyen *et al*., 2009).
- Treatment delay is the time interval between actual diagnoses and starting treatment of TB (Zerbini *et al*., 2008).